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ABSTRACT

Background: Peritoneal carcinomatosis is a poor prognostic factor for patients with gastrointestinal, gynecologic, and pancreatic cancer. Cisplatin (CDDP) is one of the most effective anti-cancer agents, although its adverse effects still remain unresolved. For the treatment of peritoneal carcinomatosis with high-dose CDDP, it is necessary to design a new delivery system of CDDP that can decrease systemic toxicity and achieve a more targeted high-dose chemotherapy.

Methods: Microspheres were prepared from gelatin of a non-toxic and biodegradable material for the sustained release of CDDP. The gelatin microspheres incorporating CDDP (GM-CDDP) were injected intraperitoneally into a mouse model of peritoneal carcinomatosis; their therapeutic efficacy and adverse effects were evaluated in comparison with intraperitoneal administration of free CDDP.

Results: GM-CDDP released CDDP in the peritoneal cavity as a result of gelatin biodegradation. The mice treated with microspheres in the peritoneal cavity lived longer than mice treated with free CDDP (74±23 days vs. 40±23 days, $p<0.05$). The mice treated with GM-CDDP also lost no weight, while the free CDDP group lost approximately 20% body weight (106±5% vs. 80±7%, $p<0.001$, body weight of day 1=100%). GM-CDDP significantly decreased the nephrotoxicity and hematotoxicity of CDDP.

Conclusions: Gelatin microsphere decreased the adverse effects of CDDP and allowed high dose intraperitoneal chemotherapy with the control of CDDP. This technique of gradual local release may allow us to provide a high dose targeted intraperitoneal chemotherapy with CDDP, resulting in enhanced anti-cancer effects. These gelatin microspheres may be useful as a drug carrier for the treatment of peritoneal carcinomatosis.

Key Words: peritoneal carcinomatosis, intraperitoneal chemotherapy, drug delivery system, gelatin

INTRODUCTION

Peritoneal carcinomatosis is one of the most serious clinical problems often observed in patients with colorectal, ovarian, gastric, pancreatic, and appendiceal carcinoma.[1] Peritoneal carcinomatosis generates malignant ascites, multiple small cancer nodules, and tumor masses of various sizes. These events impair the quality of life and lead to early mortality.[1] Metastatic dissemination in the peritoneal cavity is too widespread and numerous to perform complete a resection of each tumor mass.[2] Moreover, microscopic invasion of cancer cells was often observed in cases where no cancer nodules were observed macroscopically. Therefore, it is very difficult to treat peritoneal recurrence of cancer, even if after a complete macroscopic resection.

Intraperitoneal chemotherapy has been shown to achieve a high concentration of drugs in the peritoneal cavity in various cancers. Intraperitoneal chemotherapy decreases adverse systemic effects by maximizing the amount of drug delivered directly into the disseminated cancer cells.

Cisplatin (CDDP) is one of the most effective agents for gastrointestinal and gynecologic cancers.[3-6] Because intraperitoneal administration of CDDP transfers into the blood circulation rapidly and the retention time in the cancer tissue is very short, it is difficult to expect both high and prolonged anti-cancer effects.[7] Furthermore, due to the adverse systemic effects, such as nephrotoxicity, myelosuppression, nausea, and emesis, oncologists have to decrease the administered dose or temporarily stop chemotherapy with CDDP.

Recently, new drug delivery systems have been explored extensively to achieve greater concentrations of drugs in the cancer tissue and their controlled release for extended time periods. Most research regarding anti-cancer drugs with liposomes,[8, 9] polymeric micelles,[10] and microspheres[11-13] have designed the drug delivery based on the enhanced permeability retention effect using intravenous administration to target the cancer tissue,[14] but, such approaches are not always suitable for the treatment because of less intratumoral concentration of drugs and their concerns about systemic biosafety for clinical use. Therefore, it is important to design a novel CDDP delivery system to achieve high dose intraperitoneal chemotherapy to decrease the adverse systemic effects.

In this study, biodegradable gelatin microspheres incorporating CDDP (GM-CDDP) were prepared to achieve local controlled release of CDDP into the peritoneal cavity. Microspheres were prepared from gelatin, a non-toxic, biodegradable material for sustained release of CDDP. After GM-CDDP with different biodegradabilities was administered intraperitoneally to mice models of peritoneal carcinomatosis, their therapeutic and adverse effects were evaluated.

MATERIALS AND METHODS

Chemicals

A gelatin sample with an isoelectric point of 5.0 (molecular weight = 100,000) prepared by an alkaline process bovine bone and collagenase L were supplied by Nitta gelatin, Inc. (Osaka, Japan). Glutaraldehyde and glycine were purchased from Nacalai Tesque, Inc (Kyoto, Japan). Cisplatin was purchased from Nippon Kayaku Co., Ltd. (Tokyo, Japan). Tween 80 was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Animals

Seven-week-old, Balb/c male mice (20 g body weight) were purchased from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan) and maintained under pathogen-free conditions. The experimental treatment of animals followed the Helsinki Convention (medical), and this study protocol was approved by the Animal Experiment Committee of the Institute for Frontier Medical Sciences, Kyoto University.

Cancer cells

In our preliminary experiment, we chose the colon-26 cell line because it was sensitive to CDDP (data not shown). The cells were cultured in D-MEM medium (Invitrogen, Carlsbad, CA, USA) supplemented with 15 vol % fetal calf serum (FCS) (Thermo Inc., Waltham, USA), penicillin (50 U/ml), and streptomycin (50U/ml) and cultured at 37°C in a 95% air, 5% carbon dioxide atmosphere.

Preparation of GM-CDDP

Gelatin microspheres were prepared by chemical cross-linking of gelatin in a water-in-oil emulsion state according to the method reported previously.[15] Briefly, an aqueous solution (20 ml) of 10 wt % gelatin was preheated at 40°C and then added dropwise into 600 ml of olive oil (Wako Ltd, Osaka, Japan) at 40°C, followed by stirring at 200 or 400 rpm for 10 min to prepare a water-in-oil emulsion. The emulsion temperature was decreased to 4 °C for the natural gelation of gelatin solution to develop into non-crosslinked gelatin microspheres.

The resulting microspheres were washed three times with cold acetone in combination with centrifugation (5,000 rpm, 4°C, 5 min) to completely exclude any residual oil. Then they were fractionated by size using sieves with apertures of 20, 32, and 75 μ m (Iida Seisakusho Co. Ltd., Osaka, Japan) and air-dried at 4°C. We prepared four types of gelatin microspheres, numbered Gel 1 to 4. The non-crosslinked and dried gelatin microspheres were placed in 0.1 wt % Tween 80 aqueous solution containing different amount of 25 wt % glutaraldehyde (20 μ l to Gel 1, 50µl to Gel 2, 100µl to Gel 3 and 4) and stirred for 4 h (Gel 3) and 24 h (Gel 1,2,and 4) to allow gelatin to crosslink. After collection by centrifugation (5,000 rpm, 4°C, 5 min), the microspheres were agitated in 10 mM aqueous glycine solution at 37°C for 1 h to block the residual aldehyde groups of unreacted glutaraldehyde. The resulting microspheres were finally washed three times with double-distilled water by centrifugation, and then freeze-dried.

The CDDP powder was dissolved in double-distilled water at a final concentration of 2 mg/ml to obtain free CDDP solution. To prepare GM-CDDP, 50 µl of free CDDP solution was added to 5 mg of freeze-dried gelatin microspheres left for 24 h at 4°C.

Characterization of gelatin microspheres

Crosslinked gelatin microspheres were imaged using scanning electron microscopy (Model S-450, Hitachi, Ltd. Tokyo, Japan) after platinum coating. The gelatin microspheres were also imaged using light microscopy (CKX41, Olympus, Tokyo, Japan) in the dispersion state.

Microscopic photographs of gelatin hydrogel microspheres in the dried or water-swollen state were taken to measure their size (100 microspheres per each sample) (Figures 1C & D). The water content of the microspheres was computed based on the microsphere volume calculated from the size, which is defined as the volume percentage of water to swollen gelatin hydrogel microspheres.[16]

Evaluation of in vitro degradation of gelatin microspheres

Gelatin microspheres (10 mg) were fully swollen in 750 µl of double-distilled water for 12 h at 25°C, then 750 µl of 2N HCl aqueous solution was added and incubation took place at 25°C for various time periods. The 25°C solution was centrifuged at 5,000 rpm for 5 min, while 200µl of supernatant was collected. After that, 200 µl of fresh, 1 N HCl aqueous solution was added and incubation at 25°C continued. The absorbance of supernatant collected at 260 nm was measured by ultraviolet-visible spectroscopy (DU® 800 Spectrophotometer, Beckman Coulter, Inc., Fullerton, CA); and the amount of gelatin degraded was calculated by combining a calibration curve prepared with the gelatin solution at given concentrations.

Evaluation of in vitro CDDP release from GM-CDDP

GM-CDDP were placed in 10 ml of 0.01 mol/L phosphate-buffered saline solution (PBS, pH 7.4) containing 0.01 wt % Tween 80, and the system was agitated reciprocally at 60 strokes/min in a water bath at 37°C. At 1.5, 4, and 24 h, 5 ml of the supernatant was pipetted, and immediately after that, the same volume of PBS was added. After 24 h, 500 µl of 5µg/ml collagenase L solution was added to each sample. The same collection was performed three times for 3 h.

To measure the CDDP concentration, we measured the platinum concentration of solutions sampled on a polarized Zeeman Z-8000 atomic absorption spectrophotometer (Hitachi, Ltd. Tokyo, Japan).

Preliminary evaluation of CDDP biodistribution in vivo

Gelatin microspheres incorporating 40 µg CDDP and 40 µg free CDDP were injected intraperitoneally into normal mice. Samples of blood, ascitic fluid, and kidney were collected 1.5, 4, 24, 48, and 168 h after administration. At each time point, mice were sacrificed. Their blood was taken directly from the heart with a heparinized syringe and centrifuged to obtain serum. Next, 5ml of PBS was injected intraperitoneally, a peritoneal lavage was performed, and then the ascitic fluid was collected and centrifuged (9,000 rpm, room temperature, 5 min). The platinum concentration of serum and lavage supernatant was measured with the

atomic absorption spectrophotometer. Kidneys were treated with 70 wt% nitric acid at 90°C for 2 h,the supernatant was diluted ten times and centrifuged, and the platinum concentration of supernatant was measured similarly.

In vivo inhibitory effect of GM-CDDP for tumor development

Colon-26 cells ($1x10^6$) were suspended in 1 ml of PBS and inoculated intraperitoneally to mice on Day 0. Then, on Day 1 and 4 after cancer inoculation, GM-CDDP or free CDDP were injected into the peritoneal cavity of mice at the total administration dose of 10 mg cisplatin /kg body weight. As control groups, gelatin microspheres alone (GM without CDDP) and PBS (non-treatment) were injected. In each group, the number of the mice was 5. On Day 10, mice were euthanized and every tumor mass was resected to measure their weight.

Comparison of survival time and body weight change

For comparison of survival time, we injected 20 mg CDDP/kg of GM-CDDP or free CDDP intraperitoneally into each tumor-bearing mouse. In each group, the number of the mice was 6. Then the survival time and body weight were evaluated daily after cancer inoculation. As a control group, gelatin microspheres alone and PBS (non-treatment) were injected to the mice.

Histologic observation of milky spots after gelatin microspheres administration in vivo

The histologic cross-sections of the greater omentum of mice were made two days after intraperitoneal administration of gelatin microspheres. The greater omentum was excised and fixed in 10 wt% formaldehyde aqueous solution. A pathologist determined the location of milky spots macroscopically, and prepared those cross-sections. The section was stained with hematoxylin-eosin to view on a light microscopy.

Animal tolerance study for GM-CDDP

To examine the toxicity and tolerance for GM-CDDP comparing with free CDDP, normal mice were injected with GM-CDDP or free CDDP at a dose of 5 mg CDDP/kg weekly. As control groups, gelatin microspheres alone and PBS (non-treatment) were injected in the same way. In each group, the number of the mice was 5. The body weights of the mice were measured weekly and expressed as a percentage of their initial body weight. After five administrations, blood was taken from their hearts. The adverse effects of CDDP administration were evaluated by body weight loss, the number of red blood cells (RBC) and white blood cells (WBC), platelets (Plt), hemoglobin (Hb), and the serum concentrations of blood urea nitrogen (BUN) and creatinine (Cr). The blood samples were analyzed by FALCO biosystems Ltd. (Kyoto, Japan).

Statistical analysis

All results are expressed as the mean ± standard deviation (SD) and the data analyzed by using SPSS 6.1 software (Statistical Products and Service Solutions, Chicago, IL). Comparisons between two groups were carried out using a two-tailed, Student’s t test, and between multiple groups using one-way analysis of

variance (ANOVA) with Tukey's test of significance between individual groups. Survival analysis was performed using the Kaplan-Meier method and analyzed by the log-rank test and Cox regression. $p < 0.05$ was considered to be significant.

RESULTS

Characterization of gelatin microspheres

Figures 1 shows the scanning electron micrographs of gelatin microspheres before (Figure 1A) and after (Figure 1B) crosslinking. The microspheres of spherical shape and the surface were smooth before crosslinking and freeze-drying (Figure 1A). A crosslinking changed the microstructures of the microspheres. A porous structure was observed on the surface of microspheres. After addition of double-distilled water, the microspheres swelled rapidly (Figures 1C and D).

In vitro experiments of gelatin microspheres incorporating CDDP

We prepared four types of gelatin microspheres, and the Gel 4 microspheres with greater crosslinking showed less degradation. The other groups of microspheres degraded faster, although the degradation profile was similar (see appendix, online version only). In the CDDP-releasing test, Gels 1, 2, and 3 microspheres showed similar release profiles, and the initial burst release was around 25 to 30%. Gel 4 microspheres showed a lesser initial burst in CDDP release, and they remained longer than those of the other 3 gels. After addition of collagenase L, CDDP was released rapidly due to the degradation of gelatin microspheres. Gel 4 microspheres also showed less release than those of others (see appendix, online version only).

Based on these release data, Gel 4 microspheres were chosen for the following experiments because of the longer CDDP release profile.

CDDP biodistribution

We examined the time profile of CDDP concentration in the ascitic fluid, serum, and kidney parenchyma after the administration of GM-CDDP and free CDDP into mice (see appendix, online version only). In the free CDDP group, the Pt level in the serum and ascites was much greater in the initial 4 h than that of GM-CDDP as seen in PBS, and thereafter decreased rapidly. A high CDDP concentration was detected in the kidney in the free CDDP group even one week after administration, while the CDDP concentration remained at a lesser level in the GM-CDDP group. In contrast, the CDDP was detected in the ascites over the range of 168 h in the GM-CDDP group, whereas it decreased rapidly in the free CDDP group.

Inhibitory effect of GM-CDDP for tumor development

Figure 2A shows intraperitoneal appearance of Colon-26 tumor mass after intraperitoneal administration of GM-CDDP (a), free CDDP (b), GM without CDDP (c), and PBS (non-treatment) (d). For the free CDDP group, a few tumor nodules were detected in the abdominal cavity, but the intestinal wall was thinner and

edematous compared with that of the other groups. In the GM-CDDP groups, microspheres were still observed along the greater omentum, subphrenic area, pouch of Douglas, and hepatic portal region, and no gross tumor nodules were observed in the abdominal cavity. The tumor weights of GM-CDDP group, free CDDP group, GM without CDDP and PBS group are 108±1.45 mg, 151±66.0 mg, 1070±635 mg and 869±452 mg respectively. The tumor weight was less in the GM-CDDP and free CDDP groups compared with control groups ($p<0.001$). No statistical difference in the tumor weight was detected between the GM-CDDP and free CDDP groups (Figure 2B).

Survival time and body weight change

Figure 3A shows the change of body weight in the 2 weeks after cancer inoculation and administration. The administration of free CDDP decreased the body weight of mice, and the extent of body weight loss was greater than that of the GM-CDDP group (80±7% vs. 106±5%, $p<0.001$, BW of day 1=100%). In the both control groups, body weight loss was not observed.

Figure 3B shows the survival curve of cancer-bearing mice after the treatments. Even though free CDDP demonstrated better survival time than both control groups, the administration of GM-CDDP showed a prolonged survival time compared with that of free CDDP (74±23 days vs. 40±23 days, $p<0.05$).

Animal tolerance study

Figure 4A gives the time profile of body weight changes after the administration of GM-CDDP, free CDDP, GM without CDDP, and PBS (non-treatment). The body weight of mice decreased gradually only in the free CDDP group, whereas the GM-CDDP group and the two control groups did not show any decrease in body weight. The serum BUN and Cr levels of the free CDDP group were significantly greater than those of GM-CDDP group (BUN: 59±12 mg/dl vs. 21±2 mg/dl, $p<0.001$; Cr: 0.4±0.1 mg/dl vs. 0.2±0.1 mg/dl, $p<0.05$). The number of WBC was significantly less in the free CDDP group than the GM-CDDP group (3.6±1.3 $x10^3/\mu l$ vs. 9.9±1.7 $x10^3/\mu l$, $p<0.01$) and the control groups (Figure 4B).

Histologic observation after the application of gelatin microspheres

Figure 5 shows the histologic cross-sections of milky spots at greater omentum two days after receiving gelatin microspheres. Microspheres and cancer cells were observed around the milky spots.

DISCUSSION

In the present study, we demonstrated that biodegradable GM-CDDP was effective in decreasing the concentration of CDDP in the serum and kidney, resulting in a decrease in adverse systemic effects of CDDP when delivered via gelatin microspheres intraperitoneally. We also showed that the administration of GM-CDDP intraperitoneally in a model of peritoneal carcinomatosis in mice prolonged survival time compared with that of free intraperitoneal administration of CDDP.

Gelatin is a biodegradable biomaterial that has been extensively used for medical, pharmaceutical, and cosmetic applications. Hydrogels of different shapes can be formulated, and their feasibility as cell culture substrates,[17-19] cell scaffolds for tissue regeneration,[20-22] and carriers of growth factors or drugs[23-30] have been demonstrated experimentally. Based on the availability and ease of formulation of hydrogels, gelatin was used as the hydrogel drug carrier both for hydrophilic anti-cancer agents and hydrophobic drugs such as paclitaxel by using a miceller reaction.[31]

Gelatin is a protein with a random-coiled structure comprising 18 different types of linked amino acids. There is a chelating interaction between the carboxyl groups and CDDP molecule, although the extent depends on the polymer type.[32, 33] According to our data, CDDP is released from the hydrogel based on the hydrogel degradation. Through slower degradation of hydrogels, the in vivo volume of CDDP remains large for a longer time period. In addition, the procedure of incorporating CDDP into the hydrogel is simple without any loss of drug, while the CDDP dose can be changed only by altering the amount to be incorporated. Cancer cells often secrete matrix metalloproteinases (MMP), such as collagenase and gelatinase, which can proteolytically degrade gelatin microspheres to release the immobilized CDDP. With cancer cells that secrete greater concentrations of endogenous MMPs, gelatin microspheres may release CDDP more readily near the cancer site. Because of this feature, we believe it reasonable to use gelatin as a drug vehicle for cancer treatment. It is likely that hydrogels with a greater extent of crosslinking will degrade more slowly than those with a lesser crosslinking.[16] In this study, the Gel 4 microspheres were selected, because the microspheres with the longer time periods of CDDP release were more suitable for our experiments (see appendix, online version only).

Previous work suggests that the maximum tolerated dose of CDDP for mice ranged from 5 to 10 mg/kg, which corresponds to 100 μg to 200 μg per mouse.[34-36] Also, prior work showed that even dose-dense treatment with a maximum tolerated dose of cisplatin did not result in complete cancer eradication.36 Our preliminary experiment revealed that the tumors were already formed 48h after the implantation, and the administration of CDDP at doses of less than 100 μg did not show any significant anti-cancer effect against the non-treatment group (data not shown). Even though the administrations were done at day1 and day4, our results suggest that our model in this study was appropriate as a model of peritoneal carcinomatosis and was difficult to treat with conventional dosage of CDDP, as the situation usually faced in clinical cases.

We believe that the side effects of free CDDP might have caused the first death (Figure 3B). The mouse that died within 10 days did not exhibit any tumor nodules in the abdominal cavity at the autopsy. In contrast, the other 5 mice that died more than 25 days after the treatment, demonstrated tumor nodules and hemorrhagic ascites. We believe that some of the 5 mice may have died not only from the tumor but also of

the adverse effects of CDDP. We also thought that the initial high concentration of CDDP after the free CDDP administration killed many cancer cells, but the rapid excretion of CDDP resulted in only a transient effect of this drug to suppress cancer growth. In contrast to free CDDP administration, the GM-CDDP did not increase the initial CDDP concentration due to the controlled release of CDDP. Even though the tumor volumes of the two treatment groups were similar at day 10 (Figure 2 A & B), the survival time was different between the two treatment groups.

Because the anti-cancer activity of CDDP depends on both the dose and exposure time,[37] sustained release of CDDP is one of the superior anti-cancer effects of GM-CDDP; this release system appeared to decrease the adverse effect of CDDP, allowing a more effective prolonged means of delivering CDDP chemotherapy.

The microsphere release system can facilitate a safer and greater dose regimen of CDDP. The tolerance study with weekly administration of GM-CDDP and free CDDP support this possibility (Figure 4). The GM-CDDP significantly decreased not only the weight loss of mice, but also nephrotoxicity and hematotoxicity. The GM-CDDP did not show an initial greater peak of CDDP concentration in the kidney and serum (see appendix, online version only), which may explain the decrease in systemic toxicities without affecting the anti-cancer effect of CDDP (Figure 2).

There have been reports about drug delivery systems with CDDP nano-particles, such as liposomes or polymeric micelles, for intravenous administration on the basis of an enhanced permeability retention effects [14]; some nano-particles however, showed greater systemic toxicity than free drugs.[38] This finding may result from a much greater blood AUC of these types of drug delivery systems with CDDP nano-particles. It is conceivable that the prolonged blood levels of drugs may increase the possibility of adverse effects of anti-cancer agents. In addition, gelatin has a long history of clinical usage with established safety in contrast to other polymers[23]; the safety of some polymers as drug vehicles and their degradation products in blood circulation are unknown clinically.

The lymphatics in the omental milky spots take part in the adsorption of various substances, including cancer cells and micro-particles from the peritoneal cavity.[39, 40] Moreover, the omental milky spots are sites where cancer micrometastasis is initiated, even though the omentum appears normal on visual examination. As shown in Figure 5, the particles of the GM-CDDP were localized in the milky spots of peritoneal cavity by microscopy. Additionally, the microspheres were seen macroscopically along the greater omentum, subphrenic area, pouch of Douglas, and hepatic portal region where tumor metastases are commonly observed. This findings suggests that through the degradation of microspheres, a high concentration of CDDP was released locally near the metastatic lesions, where the concentration of MMP, gelatinase, and collagenase may be greater than in non-cancerous lesions.[41] This system can be used as an active targeting tool for peritoneal carcinomatosis.

Our study has several limitations; first, only animal experiments were carried out in this study. Second, the number of animals was limited. Third, there might be an argument about the tumor weights measurement, because it is impossible to excise all tumor cells. We removed as many tumor nodules as possible, and we thought that we were able to measure accurately the weight of all of the tumors. We are planning to examine other types of cancer, animals, and drugs, and then proceed to a clinical trial, which might have an

important impact on the treatment of patients with peritoneal carcinomatosis. We emphasize that this gelatin hydrogel release system appears to be a promising tool for the patients with peritoneal carcinomatosis.

Figure legend

Figure 1 Micrographs of gelatin microspheres.
(A) before crosslinking, (B) after crosslinking.
(C) Dry gelatin microspheres, and (D) after swelling.

Figure 2
(A)
Intraperitoneal findings of mice treated with GM-CDDP (a), free CDDP (b), GM without CDDP (c) and PBS (non-treatment) (d). Arrows indicate the nodules of cancer growth.
(B)
The total weight of cancer of mice treated with GM-CDDP, free CDDP, GM without CDDP, and PBS (non-treatment).
* $p < 0.001$, significant between the two groups. (n=5)

Figure 3
(A)
Body weight of mice during the first 2 weeks after the intraperitoneal administration of GM-CDDP, free CDDP, GM without CDDP and PBS (non-treatment).
(B)
Survival of mice after the intraperitoneal administration of GM-CDDP, free CDDP, GM without CDDP and mice without treatment (PBS).

Gelatin microspheres incorporating CDDP (○), free CDDP (△), GM without CDDP (●),and PBS group (non-treatment) (□) (n=6)

Figure 4
(A)
Bodyweight of mice receiving weekly administration of GM-CDDP (○), free CDDP (△), GM without CDDP (●) and PBS group (non-treatment) (□). (n=5)
(B)
Blood values of mice receiving weekly administration of GM-CDDP, free CDDP, GM without CDDP and PBS.
* $p < 0.05$, significant between the four groups. (n=5)

Figure 5
Histologic section of milky spots in the mouse greater omentum, 1 day after the intraperitoneal administration of gelatin microspheres.
(*) indicates gelatin microspheres, and arrows indicate cancer cells.

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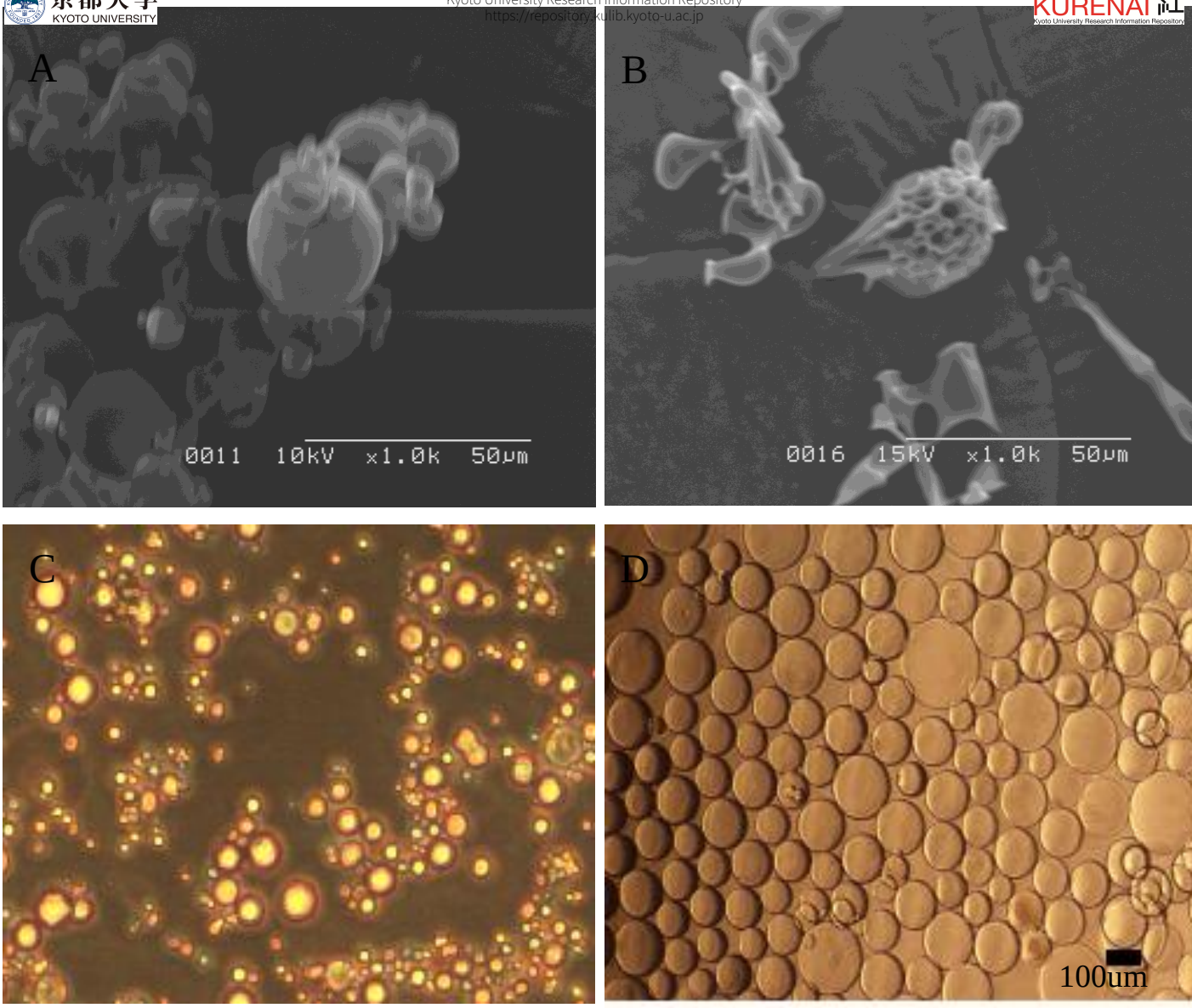

Figure 1

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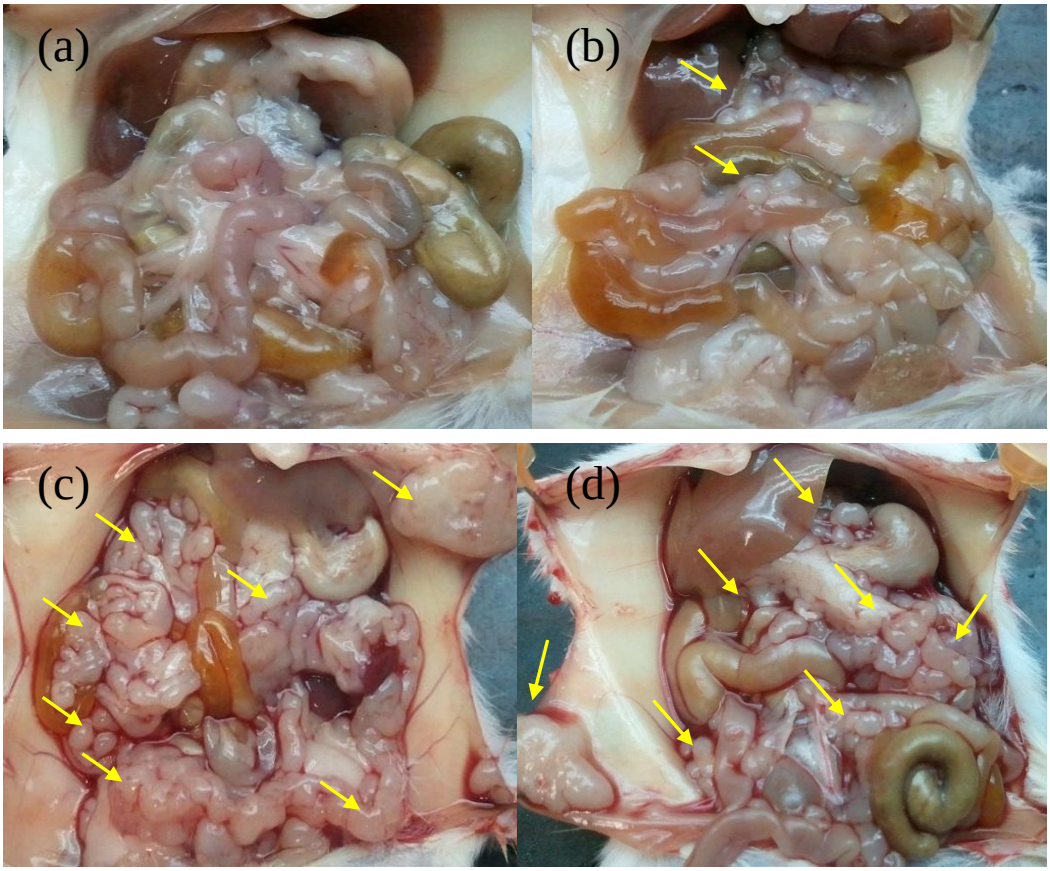

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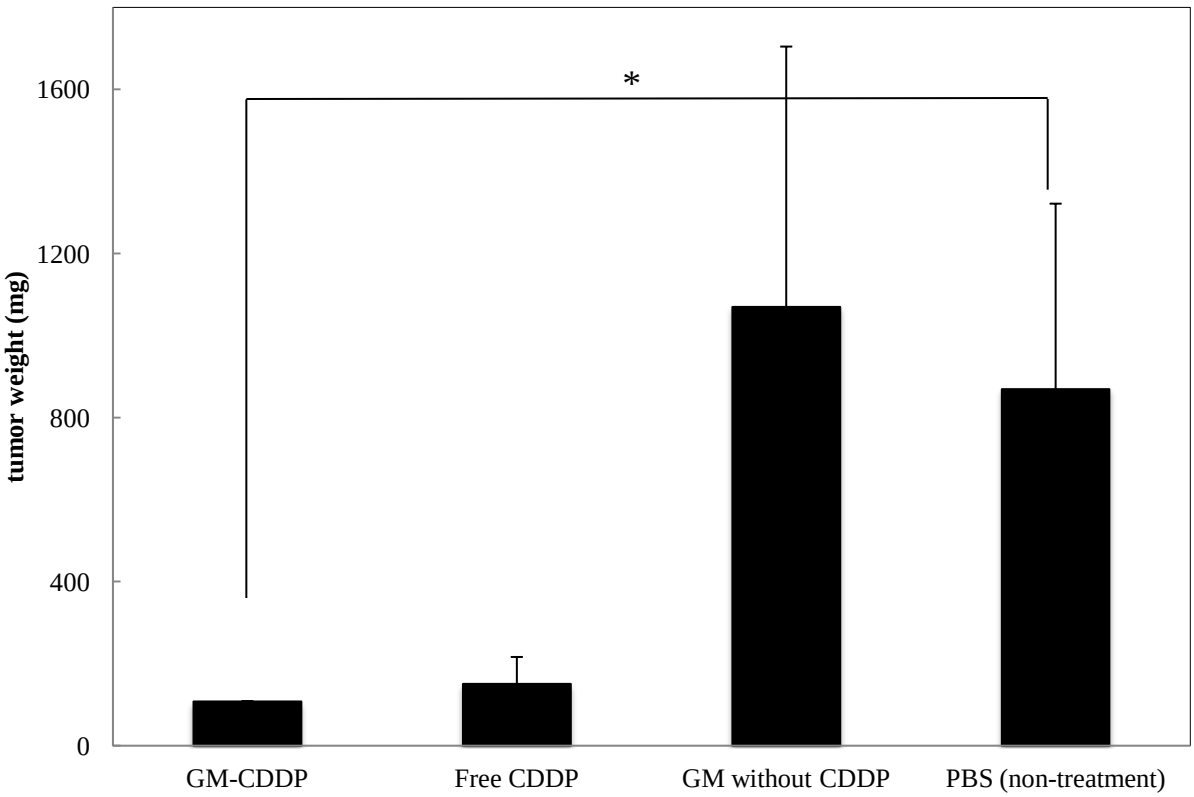

Figure 2

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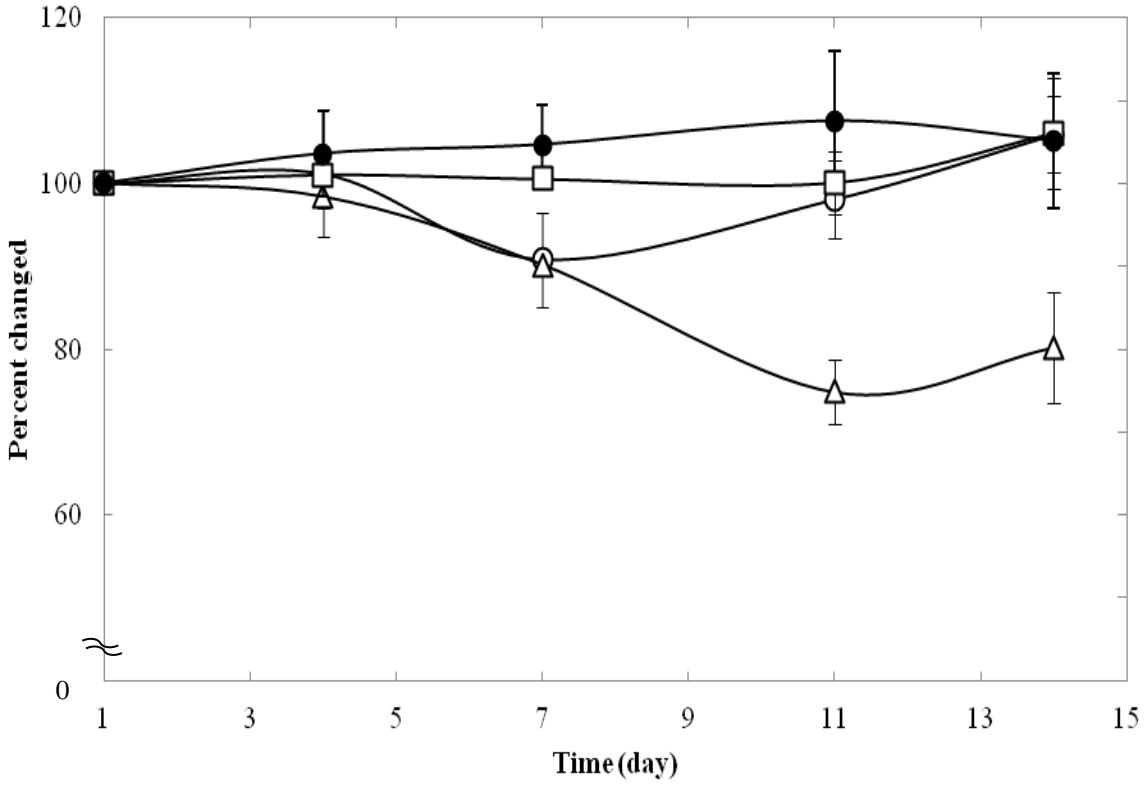

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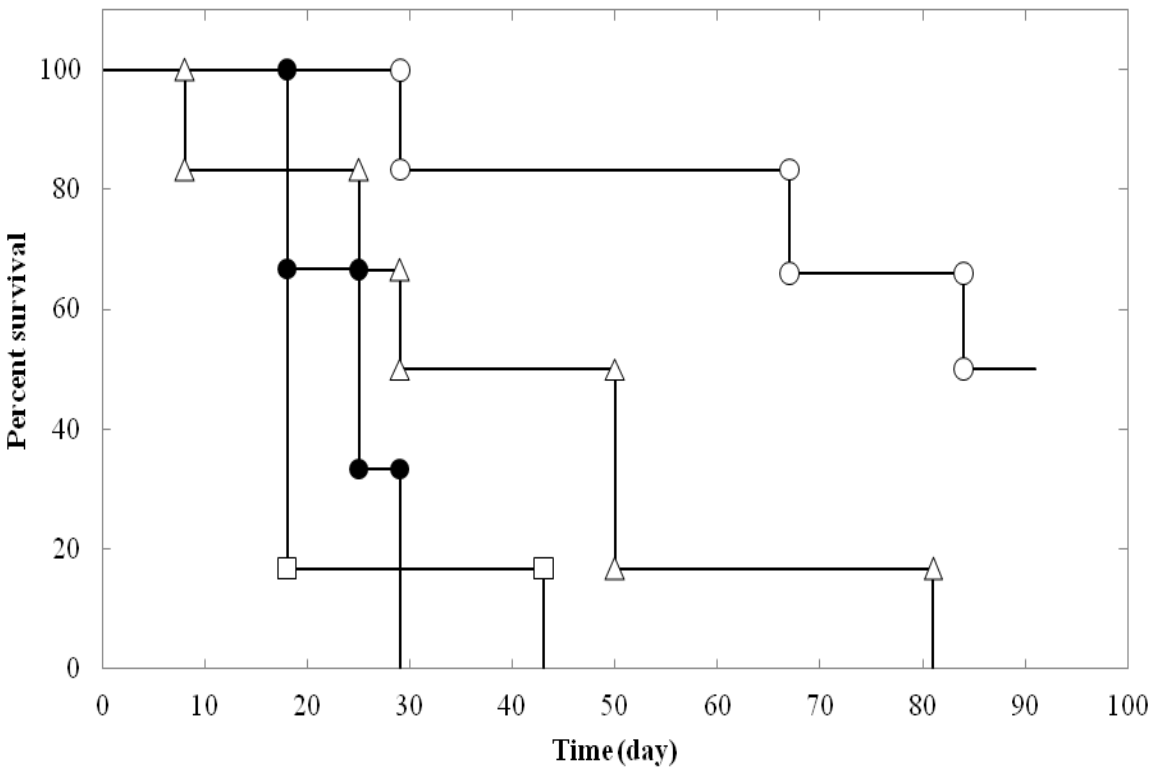

Figure 3

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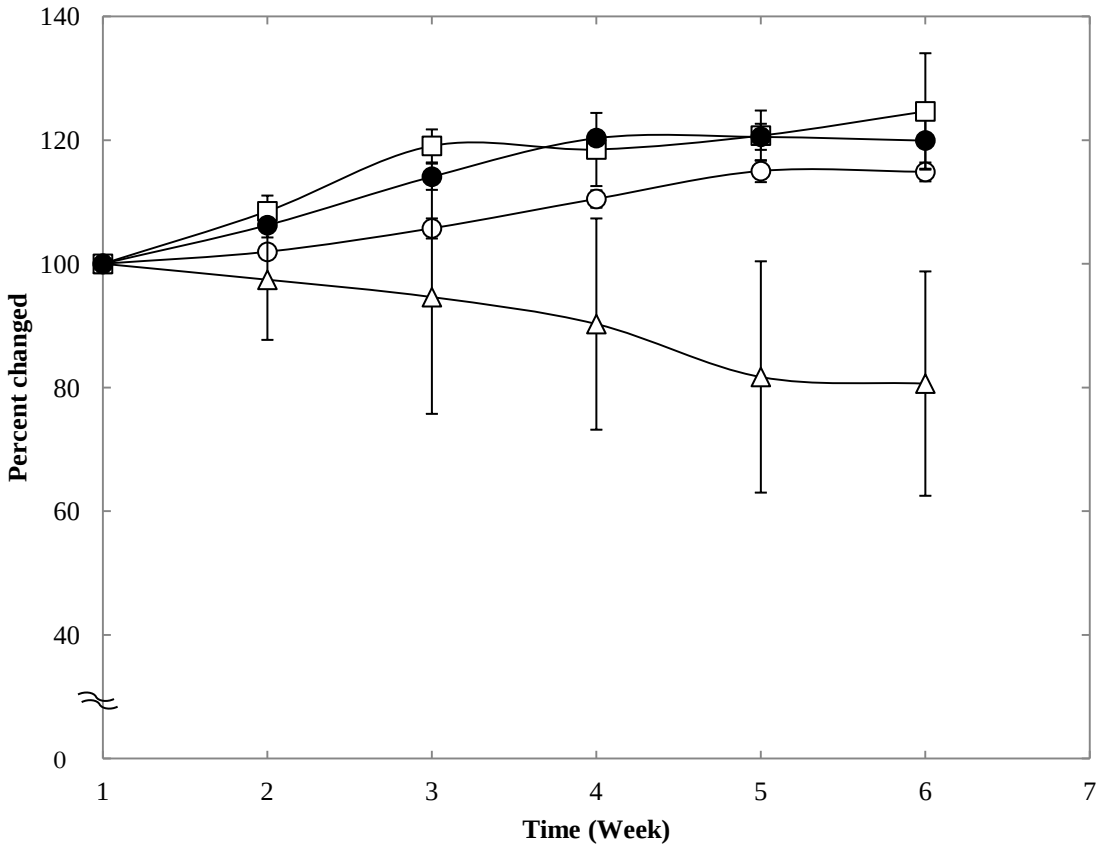

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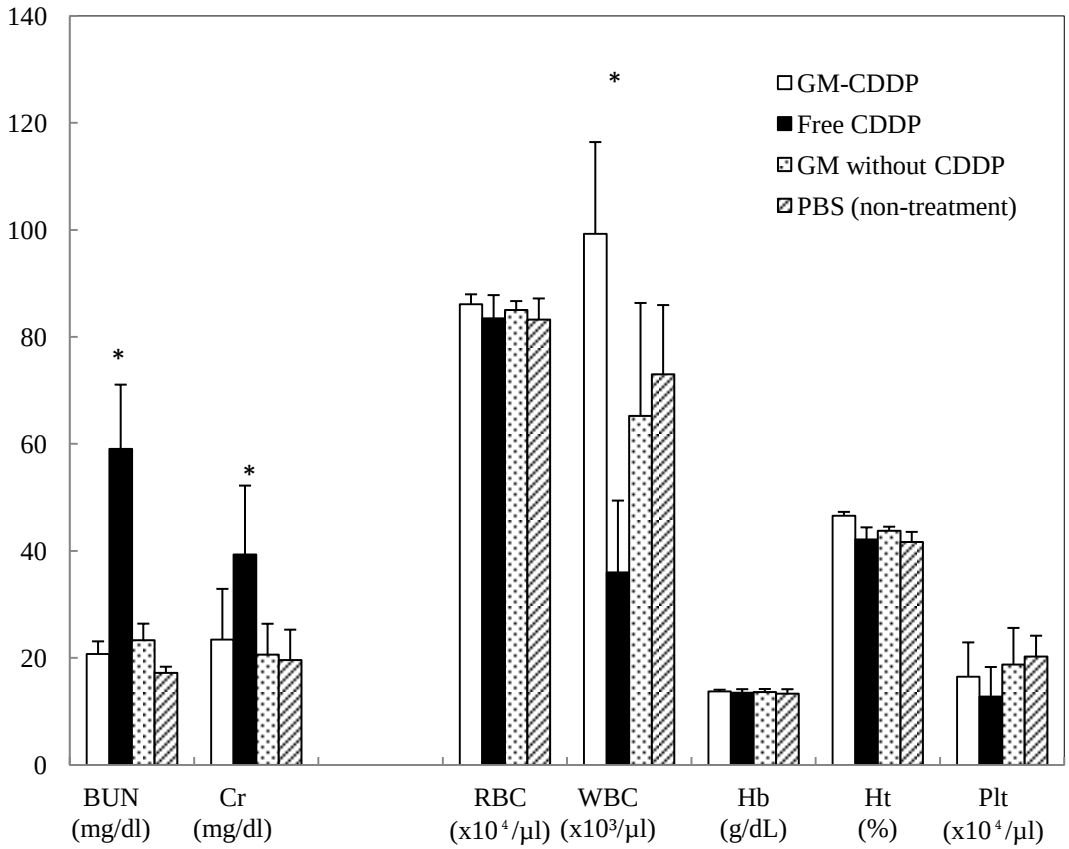

Figure 4

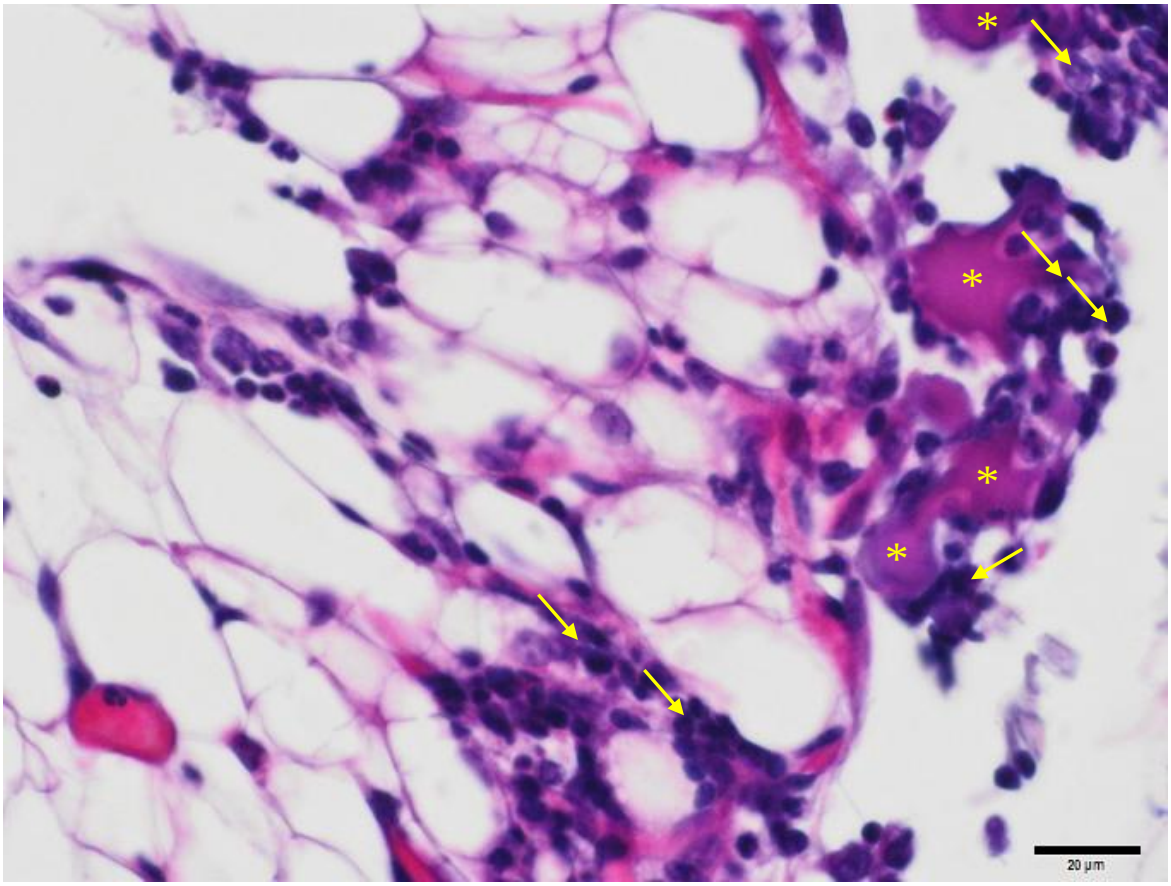

Figure5